

TAXONOMY OF PHILIPPINE SARDINES REVEALED BY BIOMETRICS DATA

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ABSTRACT

The Philippine sardines (Genus *Sardinella*, Family Clupeidae) are interesting organisms due to their great morpho-anatomical similarities. They usually thrive in marine environment with the exception of the freshwater sardine *S. tawilis* endemic to Lake Taal. To elucidate the relationship of the Philippine *Sardinella* spp., 35 biometric variables were determined and then subjected to descriptive (mean, standard deviation, and statistical range) and inferential (Factor Analysis (FA), Discriminant Function Analysis (DFA), and Hierarchical Cluster Analysis (HCA)) tests. Thirty variables correlated to the four-factor data reduction in FA that explains 96.9% of the total variance among the six sardines. In DFA, only 29 variables were found useful in differentiating the sardines. The same 29 features were able to properly predict the identity of 99.58% of the sardines. The close relationship of *S. albella* to the Lake Taal sardine was demonstrated by all three dendograms drawn from all 35 variables, only from the 30 FA informative variables, and from the 29 discriminating variables, respectively. The dendograms also show that the two populations of *S. tawilis* form two distinct clusters consistent with evidence from molecular data. Among the marine sardines, *S. lemuru* can be regarded as the farthest relative of the freshwater sardine *S. tawilis*.

Keywords: *Sardinella*, Clupeidae, morphometrics, meristics, descriptive and multivariate tests

INTRODUCTION

Sardines are moderately small, silvery fishes that live in the shallow depths of coastal water or occasionally in estuaries. They are known for their oily flesh and their two-lobed non-functional lung (Pileggi and Thompson, 1979). In addition, sardines have thin, deciduous scales that cover their elongate, spindle shaped bodies. These fishes comprise the genus *Sardinella* that belongs to the family Clupeidae [Order Clupeiformes; Class Actinopterygii]. They are considered one of the most important food fishes in the world, providing food not only to man, but also to large marine predators and sea birds (Herald, 1961). In the Philip-

pines, there are seven species identified under genus *Sardinella*, six of which are marine species (*S. albella*, *Saal*; *S. brachysoma*, *Sabr*; *S. fimbriata*, *Safi*; *S. lemuru*, *Sale*; *S. longiceps*, *Salo*, and *S. melanura*) and the other one is a freshwater species (*S. tawilis*, *Saal*) that can only be found in Taal Lake (Conlu, 1986).

S. tawilis was formerly named *Harengula tawilis* but was now given its new name based on its morpho-anatomical similarity with the members of the *Sardinella* genus. Known to local folks as tawilis, it was given an English name of freshwater herring. Tawilis can be caught throughout the year particularly during the months of September and November using gill nets set with a float-line found at a short distance below the surface of the lake (Herre, 1927). Other members of the genus like *S. albella* (white sardinella), *S. brachysoma* (deepbody sardinella), *S. fimbriata* (fringescale sardine), *S. lemuru* (Bali sardinella), *S. longiceps* (Indian oil sardine), and *S. melanura* (Blacktip sardinella) are abundant in the Balayan Bay of the South China Sea and other bays in the country (Rau and Rau, 1980; Conlu, 1986). Unlike tawilis, all the other sardines are pelagic species inhabiting coastal waters. These are called by different names depending upon the region where they are being caught. To the locals, most of these sardines are called Tamban. Other names are also used like Tuloy and Bagabas (Tagalog region), Alupaypay (Bicol), and Chevy (Sorsogon).

Delineation of species within the genus has so far been based on morpho-anatomical features alone, which in many cases, is quite confusing because of their great similarity with each other. Taxonomists have tried to resolve this problem by using rigid criteria for discriminating species that can be derived through descriptive, multivariate, and cluster analyses (Gnanadesikan, 1977). These methods have been already been applied to facilitate fish research. Beacham (1985) succeeded in differentiating the brood lines of pink salmon using meristic and morphometric characters. The same method was used by MacCrimmon and Clayton (1985) to detect the regional home-river origin of salmon. In a trawl survey of 17 groundfish species in the Southern Adriatic Sea, biometrics has become useful in elucidating a new species of *Caelorinchus* (Ungaro et al., 2001). The phylogenetic relationship of the diverse and complex genus *Barbus* was refined using biometric features of the bones, cleithra, opercular, and pharyngeal (Escala and Miranda, 2003). Through biometrics, populations of *Cobitis simplicispina* and *C. turcica* from the Sakarya-Kizilirmak river basins, Bagilli and Saz Lake in Turkey were established as unique populations (Erk'ahan et al., 2003). Sixteen morphometric and eight meristic characters, on the other hand, contributed significantly in the differentiation of the two populations of *Sabanejewia balcanica* inhabiting two rivers in Central Croatia (Bucar et al., 2003). In sardines, Intrina (1999) has employed the use of biometrics to describe three (*Saal*, *Safi*, and *Sala*) of the seven Philippine species.

This study was performed with the general aim of resolving the relationship among the different Philippine sardines, with the exception of *S. melanura* that is difficult to find and identify, using a set of biometric characters/features.

MATERIALS AND METHODS

Collection and Identification of Fish Samples

Five Philippine sardines [*S. albellus* (*Saal*), *S. brachysoma* (*Sabr*), *S. lemuru* (*Sale*), *S. longiceps* (*Salo*), and *S. tawilis* (*Sataj*)] were collected and transported to the Science and Technology Research Center (STRC), De La Salle University (DLSU) - Manila. The first four species were bought from the Navotas Fish Port, Navotas, Metro Manila. Interview with fish dealers revealed that the fish was from Cebu City and the neighboring Batangas Bay. Two populations of *S. tawilis* (*Sata*) designated as "SataA" and "SataT" were collected from the northern- (Talisay) and the southern- (Agoncillo/San Nicholas) basins, respectively, of Lake Taal.

The identity of the sardines was ascertained using the Key to the Species of Clupeidae in the Western Pacific (Muaroe et al., 1999; Fishbase 2005).

Thirty-five (35) individuals each of pre-identified *Saal*, *Sabr*, *Sale*, and the two populations of *Sata* were used for biometrics. Only 26 individuals of *Salo*, on the other hand, were tested due to low catch of this species during the time of sampling.

Biometric Methods

A total of thirty five variables consisting of three meristic (numbers of dorsal-, pectoral-, and ventral fin-rays) and 32 morphometric variables (Table 1) were counted and measured in the sardines.

Measurements were made using Vernier caliper (Mitutoyo) calibrated to the nearest 0.01 mm. In cases wherein measurements exceeded the capacity of the caliper, a millimeter ruler was used. Bilaterally symmetrical features were measured or counted on the morphometric landmarks on the left side of the fish as shown in Figure 2 and defined in Table 2. Nomenclature for the inter-landmark distances (Table 1) followed that of Armbuster (2003).

Data Analyses

Meristic and Morphometric

Biometrics data from the five Philippine sardines were combined with the *S. tawilis* data of Introna (1999). The raw data was obtained from 236 individuals belonging to six different species. The data was subjected to different statistical test: measures of central tendency (mean, standard deviation, statistical range), multivariate tests (factor- and discriminant function analyses), and hierarchical cluster analysis.

The measures of central tendency (mean, standard deviation, and statistical range) were computed using the statistical functions in Microsoft Excel. The lower limit (LL) of the statistical range is computed as the difference between the mean and twice its standard deviation (LL = mean - 2SD). The upper limit (UL), on the other hand, is equal to the sum of the mean and twice its standard deviation (UL =

= mean + 2SD). The ranges of the meristic measurements were rounded off to the nearest whole number.

For multivariate analyses, STATISTICA (version 7.0) was used. In Factor Analysis (FA), eigenvalues and their corresponding normalized eigenvectors were derived from the correlation matrix of the data. These eigenvalue-eigenvector pairs served as the principal components (PCs) in the analysis. The principal components whose eigenvalues were greater than or equal to one ($\lambda \geq 1$) were retained as significant factors, according to Kaiser's Rule. The factor pattern was rotated using the varimax raw method to magnify the proximity of a single variable with a particular factor while minimizing its proximity to the other factors. A correlation value/factor loading of $|r| \geq 0.700$ is needed for a variable to be included in a factor.

Discriminant Function Analysis (DFA), on the other hand, was used to design a model that discriminates the different species following the forward stepwise method. The variables were added one by one in the model. The ability of the variables to differentiate the specified groups is represented by F values. An F value is the ratio of the between-groups variance in the data over the average within-group variance. If the between-group variance is significantly larger than the variance within the group then there must be significant differences between the means. In this test, measurements with an F value greater than or equal to 1 ($F \geq 1$) contribute significantly in discriminating one species from the others. However, those variables with an F value of less than 1 ($F < 1$) do not. The constructed model was then allowed to run through the data to predict each sample to its corresponding species.

Hierarchical Cluster Analysis (HCA) was performed in PCord (version 3.20) using Centroid method as the linkage rule. Centroid method is an agglomerative algorithm in which the similarity is measured as the distance between cluster centroids. The centroid of a cluster is the average value of the objects obtained in the cluster on each variable. When two clusters are combined, a new centroid is computed. Due to the enormous data available, only the descriptive mean values of the sardines were utilized in establishing the relationship of the six sardines. Three dendograms, therefore, were constructed that made use of data from all 35 variables, only the correlated variables in FA, and only the discriminating variables in DFA.

RESULTS AND DISCUSSION

Descriptive Statistics on Sardines' Biometric Data

Measurements of three meristic and 32 morphometric variables from a total of 236 individuals belonging to 6 different species of Philippine sardines comprised the entire raw data. With the exception of *Sayi* data (kindly provided by Ms. Marnie Grace Intrina-Sonico), the rest of the measurements for the 2 populations of *Sata*, *Saat*, *Sabt*, *Sale*, and *Salo* are generated in the present study.

The mean, standard deviation, and statistical range were computed in all the meristic (Tables 3 to 5) and morphometric data (not shown). Table 3 presents a comparison of the sardines' dorsal fin ray counts obtained from different studies. In the present study, the dorsal fin rays of sardines ranges from 12 to 19. This value somehow fits in the 13 to 21 statistical range previously reported (Whitehead, 1985).

The pectoral fin rays of sardines range from 8 to 17 (Table 4). *S. albelia* and *S. lemuru* have 14 to 16 fin rays, whereas, the counts for *S. tawilis*, *S. brachysoma*, and *S. fimbriata* range from 8 to 15. Due in the relative wide range pectoral fin ray counts in sardines, the number of pectoral fin rays may not be a good criterion for distinguishing the different sardines. Whitehead (1985) also did not include pectoral fin rays as a variable in his study.

Another meristic character/variable considered for sardine identification was the ventral fin ray count. The mean with standard deviation, as well as, the statistical ranges of the ventral fin rays of sardines are presented in Table 5. Whitehead (1985) reported that sardines have either 8 (*S. albelia*, *S. brachysoma*, *S. fimbriata*, and *S. tawilis*) or 9 (*S. lemuru*) ventral fin rays. Results on *S. brachysoma* (8 ventral fin rays) and *S. lemuru* (9 ventral fin rays) further confirmed Whitehead's findings (1985) suggesting that the number of ventral fin ray can be used to distinguish *S. lemuru* from the rest of the sardines studied. The data of Inurina (1999) on the ventral fin ray counts of *S. tawilis*, *S. albelia*, and *S. fimbriata*, on the other hand, yielded a range of 6 to 9 ventral fin rays.

Overall, the descriptive statistics for the 32 morphometric variables were not that informative. From these values alone, it is quite difficult to ascertain which morphometric variables can actually be useful in distinguishing the different sardines. Hence, multivariate tests were conducted.

Factor- and Discriminant Function Analyses on Sardines' Biometric Data

In FA, four factors were found to satisfy Kaiser's rule (eigenvalue of ≥ 1) which means that the 35 variables can now be explained in terms of four smaller groups of variables, otherwise called as factors. These factors were retained as the principal components (PCs) in the Principal Component Analysis (PCA) which account for a total of 96.9% of the total variance observed among the six sardines.

Rotation of variables using varimax method yielded the variables correlated to each of the four factors previously identified. A variable must have correlation value/factor loading of >0.700 to be correlated to a given factor. A complete listing of variables correlated to Factors 1, 2, 3, and 4 is given in Table 6. Seventeen, eight, four, and one, variables, respectively, correlated to Factors 1, 2, 3, and 4. The remaining five variables (P, hDe, ocDH, mL, and Lcp) were found to have no substantial contribution to the total variance present among the samples. Therefore, these variables were not correlated to any of the four factors. Since all variables under each factor are highly correlated, it is therefore possible to consider

only one variable under each factor and still give a similar variance when all variables were taken together.

In DFA, 29 variables were found useful in differentiating the five sardines (Table 7). All these variables have F values greater than or equal to 1 ($F \geq 1$). The rest of the variables (paD, v-aD, V, dFL, Lvf, Lpf) have F values less than 1 ($F < 1$), thus, were excluded in the analysis. The 29 discriminating variables were then used in the forward 29-stepwise Discriminant model for sardines. Each step in the model is characterized by the addition of the variable with the highest contribution in discriminating between the specified groupings (in this case, by species).

As shown in Table 8, the model predicted the identity to the species level of 99.58% (235 out of 236 individuals) sardines. The model works perfectly (100%) in classifying *Sata*, *Sabr*, *Safl*, *Sale*, and *Salo*. The only misclassification was observed in *Safl*105 which the model identified as *SataT*.

Relationships of Sardines Revealed by Biometrics Data

The three dendograms constructed based on (1) all 35 biometric variables, (2) 30 highly correlated variables, and (3) 29 discriminating variables only gave similar topology and is therefore presented as one consensus tree (Figure 3). The sardines are distributed into five major branches [(*SataA*, *SataT*, and *Saaf*), (*Sabr*), (*Safl*), (*Sale*), and (*Salo*)]. What is interesting in this clustering is that *S. albella* (*Saaf*) grouped with the two populations of *Sata*. This grouping supports the previous findings based on molecular data that *S. albella* is the most likely ancestor to the Taal Lake sardines (Samonte *et al.*, 2000) with *S. lemuroides* as the farthest relative. Also, the separation of *SataA* and *SataT* populations into two distinct groups indicates genetic differentiation that at this point can already be observed morphologically. The differences may have developed in each of the populations due to reduced fish migration across a shallow barrier in the middle of the lake.

Conclusion

The relationship of Philippine sardines was determined using biometrics. A total of 35 biometric variables (3 meristic and 32 morphometric) were determined on 236 individuals of sardines belonging to six different species. The biometric data were then subjected to descriptive (mean, standard deviation, and statistical range) and inferential (FA, DFA, and HCA) tests.

From the descriptive values obtained, the ventral fin ray count appears to be a promising feature that distinguishes *S. lemuroides* from the rest of the sardines congruent to earlier findings of Whitehead (1985).

In FA, the 35 variables are reduced to 30 variables only that are highly correlated to four factors (eigenvalue ≥ 1). These factors or small groups of variables account for a total of 96.9% of the total variance observed among the six sardines. Twenty nine (29) variables, on the other hand, were found useful in discriminating the Philippine sardines. These variables were used in the 29-stepwise discriminant model that correctly predicted identity of 99.58% (235 out of 236) individual sardines.

CONCLUSIONS

Finally, hierarchical cluster analysis (based on all 35 biometric variables, only the 30 highly correlated variables, and the 29 discriminating variables) revealed that phenetically, *S. albella* is the closest marine relative of the freshwater *S. tawilis* with *S. temura* as the most distant marine associate. The separation of the two populations of *S. tawilis* (*SataA* and *SataT*) is also consistent with earlier findings based on molecular data (Samonte *et al.*, 2000). Thus, supporting the hypothesis of an on-going genetic differentiation between the two populations.

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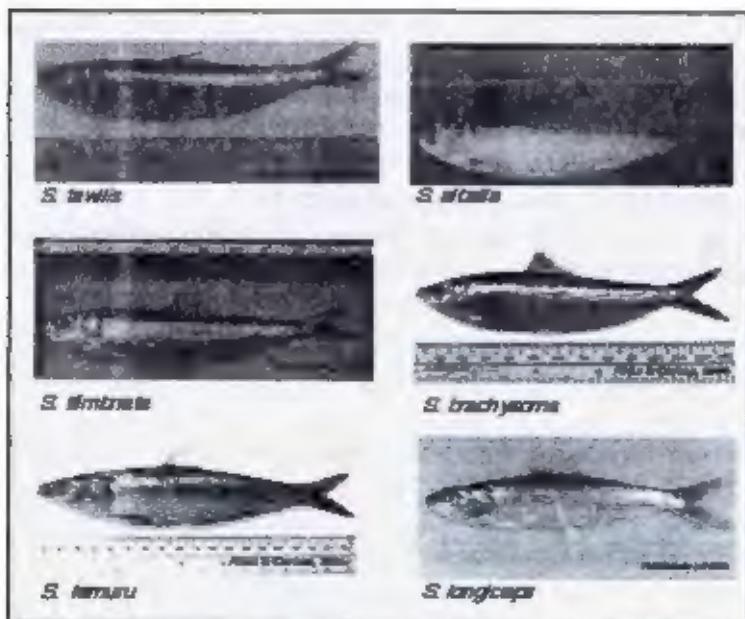


Figure 1. *Sardinella* spp. used in this study.

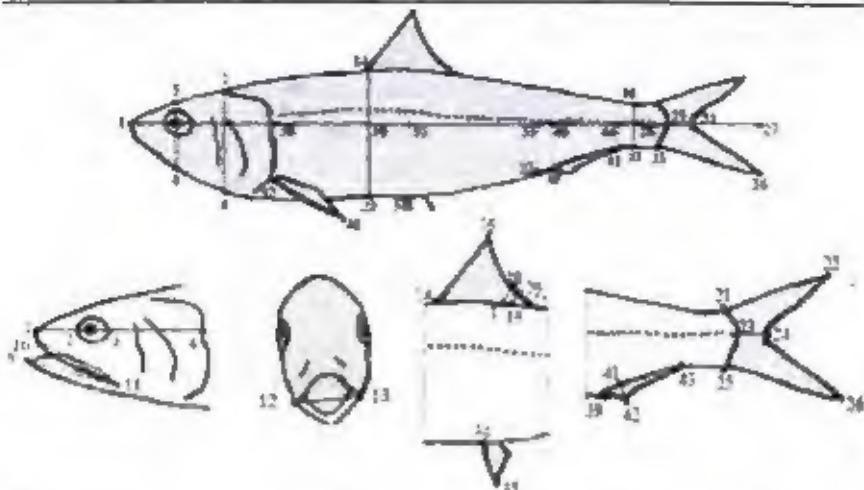


Figure 2. Landmarks and distances measured. Broken lines indicate perpendicular lines transecting the total body length (1-27) from their corresponding point of reference. See Table 1 for the names of the selected morphometrics and Table 2 for definitions of morphometric landmarks. Constructed based on Armbruster, 2003. (Drawn by K. Alvia).

Distance (Objective Function)				
32.431	1430.431	2828.430	4226.429	5624.429
Information remaining (%)				
100.000	75.000	50.000	25.000	.000



Figure 3. Representative dendrogram showing the relationship of the different sardines using Centroid linkage rule method.

Table 1. Morphometric variables used in this study. Landmarks represent the two points from where measurements were made in Fig. 2. Enclosed in parentheses are abbreviations of the variables.

Landmark	Variable
1-2	pre orbital length (preOL)
2-3	horizontal diameter of the eye (hDe)
3-4	post orbital length (posOL)
1-4	head length (hL.)
5-6	orbital depth of the head (orDH)
7-8	occipital depth of the head (ocDH)
9-11	lower jaw length (ljL)
10-11	maxillary length (mL.)
12-13	maxillary width (mW)
14-15	dorsal fin base length (dfL)
14-16	length of the first ray of the dorsal fin (L1adf)
17-18	length of the fourth ray from the posterior end of the dorsal fin (L4pdf)
19-20	length of the third ray from the posterior end of the dorsal fin (L3pdf)
21-22	length of the upper lobe of the caudal fin (Luclf)
23-24	length of the middle lobe of the caudal fin (Lmlcf)
25-26	length of the lower lobe of the caudal fin (Llclf)
1-27	maximum total length (tL.)
1-24	fork length (fL.)
1-28	maximum standard length (sL.)
14-29	maximum body depth (maxbd)
30-31	minimum body depth (minbd)
1-33	preanal distance (paD)
1-35	preventral distance (pvD)
1-36	predorsal distance (pdD)
35-37	pectoral-ventral distance (p-vD)
35-40	ventral-anal distance (v-aD)
28-40	anal-caudal distance (a-cD)
28-44	length of caudal peduncle (Lcp)
41-42	height of anal fin (Haf)
39-43	length of anal fin base (Lafb)
34-45	length of ventral fin (Lvf)
37-46	length of pectoral fin (Lpf)

Table 2. Definitions of morphometric landmarks in Figure 2.

Landmark	Definition
1	tip of mouth
2	anterior border of the left eye
3	posterior border of the left eye
4	edge of operculum
5	dorsal margin of the orbital depth of the head
6	ventral margin of the orbital depth of the head
7	dorsal margin of the occipital depth of the head
8	ventral margin of the occipital depth of the head
9	tip of lower jaw
10	tip of upper jaw
11	junction of the upper and lower jaws
12	right border of upper jaw
13	left border of upper jaw
14	anterior end of dorsal fin base
15	posterior end of dorsal fin base
16	tip of the first dorsal fin ray
17	insertion of the fourth ray from the posterior end of the dorsal fin base
18	tip of the fourth ray from the posterior end of the dorsal fin base
19	insertion of the third ray from the posterior end of the dorsal fin base
20	tip of the third ray from the posterior end of the dorsal fin base
21	insertion of the upper lobe of the caudal fin
22	tip of the upper lobe of the caudal fin
23	insertion of the middle lobe of the caudal fin
24	tip of the middle lobe of the caudal fin
25	insertion of the lower lobe of the caudal fin
26	tip of the lower lobe of the caudal fin
27	point in line # perpendicularly transecting the tip of the lower lobe of the caudal fin
28	point in line # perpendicularly transecting the insertion of the lower lobe of the caudal fin
29	ventral margin of the maximum body depth
30	dorsal margin of the minimum body depth
31	ventral margin of the minimum body depth -
32	anal opening

Table 2. (continued)

33	point in line β perpendicularly transecting the anal opening
34	anterior end of the ventral fin base
35	point in line β perpendicularly transecting the anterior end of the ventral fin base
36	point in line β perpendicularly transecting the anterior end of the dorsal fin base
37	anterior end of the pectoral fin base
38	point in line β perpendicularly transecting to the anterior end of the pectoral fin base
39	anterior end of the anal fin base
40	point in the line β perpendicularly transecting the anterior end of the anal fin base
41	proximal end of the anal fin height
42	distal end of the anal fin height
43	posterior end of the anal fin base
44	point in the line β perpendicularly transecting to the posterior end of the anal fin base
45	tip of the first ray of the ventral fin
46	tip of the first ray of the pectoral fin

Note: β , total body length

Table 3. Descriptive values on the dorsal fin ray count of five species of sardines. Ranges in the present study are computed as mean \pm 2SD rounded off to the nearest whole number.

Sardine	n	Mean \pm SD	Range		
			Present study	Intriwa (1999)	Whitehead (1985)
SardA	35	17.3 \pm 0.72	16 - 19	16-18	15-18
SardT		14.5 \pm 1.48	12 - 18		
SardL	35	15.8 \pm 1.42	13 - 19	15 - 18	13 - 21
SardR	35	16.9 \pm 0.48	16 - 18	-	13 - 21
SardF	35	17.2 \pm 0.51	16 - 18	16 - 18	13 - 21
SardE	35	15.9 \pm 0.49	15 - 17	-	13 - 21
SardO		15.4 \pm 0.58	14 - 17	-	13 - 21

Note: n, number of individuals per species; SD, standard deviation

Table 4. Descriptive values on the pectoral fin ray counts of five species of sardines. Ranges in the present study are computed as mean \pm 2SD rounded off to the nearest whole number.

Sardine	N	Mean \pm SD	Range	
			Present study	Intrina (1999)
<i>SaraA</i>	35	14.3 \pm 1.23	12-17	13-15
<i>SaraT</i>	35	12.3 \pm 2.23	8-17	-
<i>Sani</i>	35	11.7 \pm 1.73	8-15	14-16
<i>Saor</i>	35	14.1 \pm 0.37	13-15	-
<i>Safi</i>	35	14.5 \pm 0.51	14-16	13-15
<i>Sale</i>	35	15.3 \pm 0.48	14-16	-
<i>Salo</i>	26	14.8 \pm 0.73	13-16	-

Note: n, number of individuals per species; SD, standard deviation

Table 5. Descriptive values on the ventral fin ray counts of five species of sardines. Ranges in the present study are computed as mean \pm 2SD rounded off to the nearest whole number.

Sardine	n	Mean \pm SD	Present study	Range	
				Intrina (1999)	Whitehead (1985)
<i>SaraA</i>	35	7.54 \pm 0.61	6-9	{ 6-8	{ 3
<i>SaraT</i>	35	7.46 \pm 1.01	5-9	{ 6-9	8
<i>Sail</i>	35	7.63 \pm 0.33	6-9	7-9	8
<i>Saor</i>	35	8.0 \pm 0.0	8	-	8
<i>Safi</i>	35	7.66 \pm 0.48	7-9	7-9	8
<i>Sale</i>	35	9.0 \pm 0.0	9	-	9
<i>Salo</i>	26	7.69 \pm 0.47	7-9	-	8

Note: n, number of individuals per species; SD, standard deviation

Table 6. Summary results of Factor Analysis showing reduction to four factors and their corresponding correlated variables

Factor	Correlated Variables
1	V, hL, preOL, posOL, orDH, mW, L4pdf, L3pdf, tL, fL, sL, paD, pvd, pdD, p-vD, v-aD, Lpf
2	dfL, Lulcf, Llacf, maxbd, minbd, a-cD, Lafb, LvF
3	D, ijL, Lmlcf, Haf
4	Lladf

Table 7. List of discriminating and non-discriminating variables

Variables	
Discriminating Variables	L4pdf, ijL, a-cD, minbd, Lmlcf, Lladf, Haf, posOL, maxbd, D, mW, mL, LaFB, Lcp, p-vD, tL, orDH, fL, Lulcf, hL, pdD, ocDH, pvd, hDe, 3pdf, paD, preOL, P.Llacf, SL
Non-discriminating Variables	paD, v-aD, V, dfL, LvF, Lpf

Table 8. Discriminant Function Analysis predictions (%) for the correct identification of Philippine sardines.

Observed sardine	n	Number of individuals predicted							% correct prediction
		SardA	SardT	Sagi	Sabr	Safi	Sale	Salo	
SardA	35	35	0	0	0	0	0	0	100.00
SardT	35	0	35	0	0	0	0	0	100.00
Sagi	35	0	41	34	0	0	0	0	97.14
Sabr	35	0	0	0	35	0	0	0	100.00
Safi	35	0	0	0	0	35	0	0	100.00
Sale	35	0	0	0	0	0	35	0	100.00
Salo	26	0	0	0	0	0	0	26	100.00
Total	236								99.58

Note. * = incorrect DFA-model identifications